

IN THE SPECIFICATION

Please amend the specification as follows:

Please amend the paragraph on page 4, line 1 as follows:

Fluorescent or luminescent substrates or products of enzyme reactions have been employed in protein assay multiplexing. For example, fluorescent beads having ligands for up to 15 different cytokines were employed to detect two or more different cytokines (DeJager et al., 2003) and fluorescein diphosphate and casein BODIPY-FL® were employed to detect alkaline phosphatase and certain proteases (Nolkrantz et al., 2002).

Please amend Table 2 on page 24 as follows:

Table 2

Fluorophore	Excitation (nm)	Emission (nm)
Fluorescein (FITC)	495	525
Hoechst 33258	360	470
R-Phycoerythrin (PE)	488	578
Rhodamine (TRITC)	552	570
Quantum Red QUANTUM RED™	488	670
Texas Red <u>TEXAS</u> RED™	596	620
Cy3	552	570
Rhodamine-110	499	521
AFC	380	500
AMC	342	441
Resorufin	571	585
BODIPY FL®	504	512
BODIPY TR®	591	620

Please amend the paragraph on page 27, line 14 as follows:

~~Caspase-Glo™~~ CASPASE-GLO™ 8 Reagent (~~Caspase-Glo™~~ CASPASE-GLO™ 8 Assay System, Promega, Corp.) was evaluated for its ability to allow multiplexing of homogeneous luminogenic caspase-8 and nonluminogenic caspase-3 enzyme assays. ~~Caspase-Glo™~~ CASPASE-GLO™ 8 Reagent is comprised of ~~Caspase-Glo™~~ CASPASE-GLO™ 8 Buffer and the luminogenic substrate Z-LETD-aminoluciferin. For the luminogenic assays in Figure 1A, either ~~Caspase-Glo™~~ CASPASE-GLO™ 8 Reagent (diamonds) or ~~Caspase-Glo™~~ CASPASE-GLO™ 8 Reagent also containing 50μM of the fluorogenic substrate for caspase-3, (Z-DEVD)₂-rhodamine-110 (squares), was used to demonstrate the feasibility of a multiplexed luminogenic and nonluminogenic assay. For the fluorogenic assay in Figure 1B, ~~Caspase-Glo™~~ CASPASE-GLO™ 8 Buffer containing either 50μM (Z-DEVD)₂-rhodamine-110 and 10mM DTT (diamonds) or 50μM (Z-DEVD)₂-rhodamine-110 and Z-LETD-aminoluciferin (squares) were used.

Please amend Table III as follows:

Table III

Component	1	2	3	4	5	6	7	8	9	10	11	12
<u>Caspase-Glo™ CASPASE-8</u> GLO™ 8 Buffer	400	400	400	400	400	400	400	400	400	400	400	495
<u>Caspase-Glo™ CASPASE-8</u> GLO™ 8 lyophilized substrate reconstituted in 1ml of water	100	na	na	100	100	100	100	100	100	100	100	na
5mM (Z-DEVD) ₂ -rhodamine-110	na	5	na	5	5	na	na	na	5	5	na	5
5mM Ac-DEVD-AMC	na	na	5	na	na	5	na	na	na	na	na	na
DMSO	5	na	na	na	na	na	5	5	na	na	5	na
100 mM Hepes	na	80	80	na	na	na	na	na	na	na	na	na
1M DTT	na	20	20	na	na	na	na	na	na	na	na	na
Caspase-3 inhibitor (in excess)	na	na	na	na	Yes	na	na	Yes	na	na	Yes	na
Caspase added	8	3	3	8&3	8&3	8&3	3	3	8	3	8	3

Please amend the paragraph on page 31, line 1 as follows:

The components from Table III were added to replicate wells and reactions were incubated at room temperature for two hours. The buffer employed was that from the ~~Caspase-Glo™~~ CASPASE-GLO™ 8 Assay System. DMSO was obtained from Sigma-Aldrich and the DTT was obtained from Amresco. The substrates and inhibitors were obtained from Promega Corp.

Please amend the paragraph on page 32, line 9 as follows:

Following incubation, relative luminescence for caspase-8 activity was measured using BMG Fluorostar (BMG Labtechnologies Ltd.). Relative fluorescence was determined using the Labsystems ~~Fluoroskan Ascent~~ FLUOROSKAN ASCENT™ plate reader. For caspase-3 activity, a filter set of 485_{EX}/527_{EM} was utilized. For trypsin activity, a filter set of 360_{EX}/460_{EM} was used.

Please amend the paragraph on page 32, line 24 as follows:

Reagents were prepared by reconstituting ~~Beta-Glo®~~ BETA-GLO® lyophilized substrate with ~~Beta-Glo®~~ BETA-GLO® Buffer (~~Beta-Glo®~~ BETA-GLO® Assay System, Promega Corp.), or adding (Z-DEVD)₂-rhodamine-110 (50 μM) to ~~Beta-Glo®~~ BETA-GLO® Buffer, or reconstituting ~~Beta-Glo®~~ BETA-GLO® lyophilized substrate with ~~Beta-Glo®~~ BETA-GLO® Buffer and adding (Z-DEVD)₂-rhodamine-110 (50 μM). Caspase-3 (2 μl/ml, Pharmingen Corp), or β-galactosidase (0.1 μl/ml), or caspase-3 and β-galactosidase, were diluted in RPMI 1640 and 100 μl were added to wells of a 96-well white plate. 100 μl of the appropriate reagent were added to wells of a 96-well plate and the plates were incubated at room temperature. Luminescence was measured using a DYNEX Laboratories MLX™ plate luminometer at 30 minutes. Fluorescence was measured 2 hours post incubation on a CytoFluor II Fluorescent plate reader with a filter set of 485_{EX}/530_{EM}. All measurements were repeated at 18 hours with different gain settings on the CytoFluor II fluorometer to compensate for increased fluorescence.

Please amend the paragraph on page 34, line 5 as follows:

~~Caspase-Glo™~~ CASPASE-GLO™ 3/7 Reagent (~~Caspase-Glo™~~ CASPASE-GLO™ 3/7 Assay, Promega, Corp.) which contains Z-DEVD-aminoluciferin was combined with (Z-DEVD)₂-rhodamine-110 or Ac-DEVD-AMC in the presence of caspase-3 with either a caspase-3 inhibitor (Ac-DEVD-CHO, 10 µM) or with a luciferase inhibitor (Resveratrol, 5 µM). The luminescent signal from caspase-3 cleavage of Z-DEVD-aminoluciferin was read at 30 minutes, while the fluorescent signals from caspase-3 cleavage activity were read at 2 hours using the appropriate AMC or rhodamine 110 filter sets.

Please amend the paragraph on page 35, line 6, as follows:

Sample dilutions of LDH (0, 1:8000, 1:4000, 1:2000, diamonds), ATP (0, 1.25, 2.5, and 5 µM, squares), and a combination of LDH/ATP (0/0 µM, 1:8000/1.25 µM, 1:4000/2.5 µM, and 1:2000/5 µM, respectively, triangles) were made with a 10 mM HEPES pH 7.5, 0.1% Prionex (PentaPharma Corp) solution, and 100 µl of the dilutions (n = 4) were added to wells of a white, 96-well plate. The appropriate detection reagent (100 µl) was added to the samples, the plates were protected from light, mixed for 30 seconds, and incubated at room temperature. Following an eight minute incubation, fluorescence was measured on a LabSystems ~~Fluoroskan Ascent~~ FLUOROSKAN ASCENT™ plate reader with filter set 560_{EX}/590_{Em}. At 30 minutes post-incubation luminescence was recorded using a ~~Dynex~~ DYNEX MLX™ plate luminometer.

Please amend the paragraph on page 35, line 25 as follows:

The following detection reagents were prepared: 1) LDH reagent- ~~Caspase-Glo™~~ CASPASE-GLO™ 3/7 Buffer supplemented with 238µM resazurin was used to reconstitute the CytoTox-ONE™ lyophilized substrate; 2) caspase-3 reagent-the ~~Caspase-Glo™~~ CASPASE-GLO™ 3/7 buffer was used to reconstitute the ~~Caspase-Glo™~~ CASPASE-GLO™ 3/7 lyophilized substrate as per Promega Technical Bulletin 323; 3) LDH/caspase-3 combined

reagent-LDH reagent (prepared as above) was used to reconstitute the lyophilized Caspase-Glo™ CASPASE-GLO™ 3/7 substrate. The LDH detection reagent compound of the LDH/caspase-3 combined reagent is unstable due to the presence of DTT in the Caspase-Glo™ CASPASE-GLO™ 3/7 lyophilized substrate, so this reagent was prepared immediately prior to addition to samples.

Please amend the paragraph on page 36, line 3, as follows:

Sample dilutions were prepared in 10 mM HEPES pH 7.5, 0.1% Prionex (PentaPharma Corp) solution: 0, 1:8000, 1:4000, 1:2000 dilutions of LDH (diamonds); 0, 5, 10, and 20 U/ml caspase-3 (BIOMOL Laboratories, squares), and a combination of LDH/caspase-3 (0/0 U/ml, 1:8000/5 U/ml, 1:4000/10 U/ml, and 1:2000/20 U/ml, respectively, triangles). 100 µl of the dilutions (n = 4) were added to white, 96-well plates. The appropriate detection reagent (100 µl) was added to the samples, and the plates were protected from light, mixed for 30 seconds, and incubated at room temperature. Following a six minute incubation at room temperature, fluorescence was measured on a Labsystems ~~Fluoreskan Ascent~~ FLUOROSKAN ASCENT™ plate reader with filter set 560_{EX}/590_{Em}. At 45 minutes post-incubation luminescence was recorded using a ~~Dynex~~ DYNEX MLX™ plate luminometer.

Please amend the paragraph on page 37, line 18, as follows:

Sample dilutions were prepared in 10 mM HEPES pH 7.5, 0.1% Prionex (PentaPharma Corp) solution; 0, 1, 2, and 4 U/ml PKA (diamonds), 0, 5, 10, and 20 U/ml caspase-3 (squares), and a combination of PKA and caspase-3 (0/0 U/ml, 1/5 U/ml, 2/10 U/ml, and 4/20 U/ml, respectively, triangles), and 40 µl of the dilutions (n = 4) were added to white, 96-well plates. The appropriate detection reagent (40 µl) was added to the samples, the plates were protected from light, mixed for 30 seconds, and incubated at room temperature for 20 minutes. Following incubation, 40 µl of a protein kinase stop reagent were added to the wells which contained either the kinase reagent alone or the combination kinase/caspase-3 reagent. The plates were mixed an additional 30 seconds, protected from light, and incubated for 30 minutes longer at room

temperature. Fluorescence was measured on a Labsystems ~~Fluoroskan Ascent~~ FLUOROSKAN ASCENT™ plate reader with filter set 485_{EX}/527_{Em}. Luminescence was recorded using a ~~Dynex~~ DYNEX MLX™ plate luminometer.

Please amend the paragraph on page 38, line 30 as follows:

CHO-K1 hRL25 cells were harvested and plated into a 96-well clear bottom, white walled tissue culture plate at a density of 20,000 cells/well, and incubated overnight at 37°C in 5% CO₂. Staurosporine at a final concentration of 0, 0.5, 1, 2 μM (10 μl/well) was added to the appropriate wells to initiate cell death, thus altering luciferase activity. Cells were incubated for an additional 3.5 hours at 37°C in 5% CO₂. Various concentrations of caspase-3 (BIOMOL Laboratories) were added to the appropriate wells at 0, 5, 10, and 20 U/ml, in tissue culture medium (10 μl/well). Therefore, combined staurosporine/caspase-3 concentrations for data points were 0 μM/0 U/ml, 0.5 μM/5 U/ml, 1 μM/10 U/ml, and 2 μM/20 U/ml, respectively. Immediately after addition of the caspase-3 enzyme, 10 μl/well of either luciferase substrate, caspase-3 substrate, or luciferase/caspase-3 substrates were added to the appropriate wells. After addition of the detection reagents, the plates were mixed briefly and incubated at 37°C in 5% CO₂ for two hours. Fluorescence was measured on a Labsystems ~~Fluoroskan Ascent~~ FLUOROSKAN ASCENT™ plate reader with filter set 485_{EX}/527_{Em}. Luminescence was recorded using a ~~Dynex~~ DYNEX MLX™ plate luminometer.